Response of two Antarctic bryophytes to stratospheric ozone depletion

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Received: September 2001;

Key words: Antarctica, bryophytes, maximum quantum yield of photochemistry ($\boldsymbol{F_v/F_m}$), pigmentation, stratospheric ozone (O_3) depletion, ultraviolet-B (UV-B) radiation

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Running title: Antarctic bryophytes and ozone depletion

Abstract

We report a study which measured changes to the radiative environment arising from stratospheric O_3 depletion at Rothera Point on the western Antarctic Peninsula (67° S, 68° W) and subsequent associations between these changes and pigmentation and maximum quantum yield of photochemistry (F_v/F_m) of two Antarctic bryophytes, the liverwort *Cephaloziella varians* and the moss *Sanionia uncinata*. We found a strong relationship between O_3 column depth and the ratio of UV-B to PAR irradiance (F_{UV-B}/F_{PAR}) recorded at ground level. Weaker, but significant, associations were also found between O_3 column depth and noon irradiances and daily doses of unweighted and biologically effective UV-B radiation received at ground level. Regression analyses indicated that F_{UV-B}/F_{PAR} and daily dose of unweighted UV-B were best predictors for concentrations of total carotenoids and UV-B screening pigments extracted from bryophyte tissues. Concentrations of these pigments were loosely but significantly positively associated with O_3-dependent irradiance parameters. HPLC analyses of carotenoids also suggested that both species increased the synthesis of neoxanthin during periods of O_3 depletion. Violaxanthin, lutein, zeaxanthin and β,β-carotene concentrations were also apparently influenced by O_3 reduction, but not consistently across both bryophyte species. Concentrations of chlorophylls *a* and *b* were apparently unaffected by O_3 depletion.

No direct associations between F_v/F_m and O_3-dependent irradiance parameters were found. However stepwise multiple regression analyses suggested that the production of UV-B screening pigments conferred protection from elevated F_{UV-B}/F_{PAR} on F_v/F_m in both species and that carotenoids conferred protection on F_v/F_m in *Sanionia*. Our data suggest that changes to the radiative environment associated with stratospheric O_3 depletion influence the pigmentation of two Antarctic bryophytes, but that F_v/F_m is unaffected, at least in part because of rapid synthesis of protective pigments in response to O_3 depletion.

Introduction

Man-made halocarbons released into the atmosphere have caused widespread depletion of stratospheric ozone (O_3) since the early 1980s (World Meteorological Organization 1999). Substantial O_3 reductions, arising from photochemical catalysis by reactive chlorine and bromine species on the surfaces of polar stratospheric clouds, have been recorded over Antarctica, leading to the annual springtime 'ozone hole' (Farman *et al.* 1985). Widespread, but less severe, O_3 depletion also occurs over northern and southern midlatitudes and the Arctic (Stolarski *et al.* 1992; Müller *et al.* 1997). Since O_3 absorbs biologically damaging solar ultraviolet-B (UV-B) radiation (280-315 nm), reductions in stratospheric O_3 lead to increased irradiances of UV-B received at the Earth's surface (World Meteorological Organization 1999).

Considerable effort has been invested in determining plant response to simulated O_3 depletion during the past two decades. The two main experimental approaches used have been to apply supplemental UV-B radiation to plants from fluorescent UV lamps or to filter UV-B from solar radiation, usually with plastic screens. These artificial exposures have suggested that O_3 depletion may increase foliar concentrations of UV-B screening pigments, typically flavonoids, but, in many plant species, has a negligible effect on photosynthesis and growth (Caldwell & Flint 1994; Searles *et al.* 2001). However, although these exposures have built up a generic picture of plant response to elevated UV-B, they are often confounded by methodological problems. For example, the low irradiances of UV-A radiation (315-400 nm) and PAR (photosynthetically active radiation; 400-700 nm) received by plants in laboratory or glasshouse exposures tend to produce anomolously severe responses to UV-B radiation (Caldwell & Flint 1994). The increasing use of outdoor irradiation systems in recent years has led to less exaggerated effects of elevated UV-B on plant growth. However, in non-modulated (or 'square wave') UV-B systems, in which lamps produce a constant supplement of UV-B irrespective of ambient solar radiation conditions, unrealistically high irradiances of UV-B received by plants during cloudy periods may result in anomolous effects of UV-B on plant growth (Allen *et al.* 1998). Modulated outdoor UV-B exposure systems negate this problem, since plants are irradiated with UV-B radiation from lamps against natural background levels of UV-A and PAR, but recent data indicate that some systems may yield artefactual results owing to plant response to UV-A radiation or some other factor associated with energized lamps (Newsham *et al*. 1996). Filtration experiments suffer from the problem that screens substantially alter several other abiotic factors other than incident radiation, notably temperature and precipitation (Kennedy 1995), although efforts have been made to reduce these problems (e.g. Rousseaux *et al.* 2001).

Experiments in locations experiencing substantial stratospheric O_3 depletion are therefore required to determine plant response to O_3-dependent increases in UV-B radiation. Recent studies on the Antarctic Peninsula using screens to filter UV-B from solar radiation have examined plant responses to altered UV-B radiation several weeks after treatments have been applied (Day *et al.* 1999; Ruhland & Day 2000; Xiong & Day 2001). However, although these studies have examined how Antarctic vegetation responds to medium term changes in incident UV-B, they have not addressed whether plant response is associated with daily variation in UV-B radiation associated with O_3 depletion or not. Little is known of how plants respond to O_3 depletion *per se*: Rousseaux *et al.* (1999) found a significant association between DNA damage in the foliage of the herb *Gunnera magellanica* and increasing UV-B irradiances arising from O_3 depletion over Tierra del Fuego, but, other than these data, no information exists as to how O_3 depletion influences terrestrial plants. In the light of this, we performed a non-manipulative study into the effects of O_3 depletion on the pigmentation and photosynthesis of two bryophyte species in late austral spring and early summer on the western Antarctic Peninsula, at a location *c*. 1,500 km south of Tierra del Fuego. Substantial depletion of O_3 occurs in the stratosphere over the Antarctic Peninsula at this time of year, coinciding with the emergence of plants from melting snow and ice, which results in wide variation in irradiances of solar UV-B received by vegetation. We selected bryophytes for study because the majority lack a cuticle, which can strongly absorb UV-B radiation (Krauss *et al.* 1997), and we hence anticipated significant responses to elevated UV-B radiation in these plants.

Materials and methods

Study site and sampling

Plants of the liverwort *Cephaloziella varians* (Gottsche) Steph. and the moss *Sanionia uncinata* (Hedw.) Loeske *ex* Nitardy were sampled daily at solar noon (13:30 hrs local time) between 16 November and 28 December 1998 and between 11 November and 10 December 1999 from a gully fed by meltwater from a permanent snowbank, and situated at *c*. 5 m above sea level at Rothera Point, Adelaide Island, western Antarctic Peninsula (67° 34' S, 68° 07' W; Fig. 1). Plants in the gully were exposed to solar radiation without shading between *c*. 10:00 and 19:00 hrs local time during the period of study. Snowfalls during the study were relatively infrequent, but occurred on 16-18, 23, 29 and 30 November 1999, when snow was gently cleared from plants at least 4 h before sampling. Three separate colonies of each bryophyte (*c*. 50 × 50 mm) were excised with a scalpel and, in 1998, were immediately taken in clean polythene bags in the dark to a laboratory situated *c*. 500 m from the gully. Measurements of quantum yield of photochemistry were made on colonies before taking them to the laboratory in 1999 (see *photochemical yield measurements*).

Pigment measurements

Once at the laboratory, the uppermost 2-3 mm of foliage was cut from each colony and, to facilitate grinding, was blotted on tissue paper until no free water could be drawn from the tissues (Post & Vesk 1992). Preliminary analyses, using the spectrophotometric methods detailed below, indicated that this process removed *c*. 2% of the pigments analysed for in this study from plant tissues. Additional subsamples of unblotted material were also retained for HPLC analyses in 1999 (see below). Each sample was then divided into three 100 mg subsamples. Two of the subsamples were ground separately with *c*. 1.5 g of silver sand in a mortar with a pestle for 0.5 min. To extract chlorophylls and carotenoids, 3 mL of methanol was added to the first subsample and the tissues were ground for 1 min. The extracts were then centrifuged at 6000 rpm for 10 min at 0 °C and 700 μL of these extracts were passed through 0.45 μm filters (Whatman International Ltd., Maidstone, UK). To extract UV-B screening pigments, 3 mL of a 70:20:1 mixture of methanol, water and HCl was added to the second subsample and the tissues were ground for 1 min. The extracts were heated at 65 °C for 10 min and were then centrifuged at 6000 rpm for 10 min, the supernatants removed and placed in a refrigerator at 4 °C, and a further 1 mL of the methanol, water and HCl mixture was added to the pellet, which was resuspended before heating again at 65 °C for 10 min (Post & Vesk 1992). The supernatants were combined and 700 μL of the extracts passed through 0.45

μm filters. The third subsample was dried to constant mass (60 °C for 48 h) and weighed to enable pigment data to be expressed on a per unit dry mass basis.

Extracts were transferred to UV grade polymethyl methacrylate semi-microcuvettes (PMMA; Kartell spa, Milan, Italy), diluted and absorbances were measured immediately in a spectrophotometer. To estimate concentrations of chlorophylls *a* and *b* and total carotenoids, absorbances of methanol extracts were measured at 470, 653 and 666 nm and concentrations were calculated using standard formulae (Lichtenthaler & Wellburn 1983). Masses of chlorophylls and carotenoids extracted per unit dry mass of tissue were subsequently calculated. To estimate concentrations of UV-B screening pigments, the absorbance of each acidified methanol extract was measured between 280 and 315 nm (step 1 nm). Concentrations of UV-B screening pigments were expressed in arbitary units as the area under the absorbance curve ($AUC_{280-315}$) per unit dry mass of tissue. Sample preparation and analyses were conducted in dim light and were usually completed within 2 h of excision. Pigment data were not available on 20 November 1998 for *Cephaloziella* and on 30 November 1998 for both species.

HPLC analyses were made on a subset of samples to test whether or not bryophytes increased or decreased the synthesis of specific carotenoids in response to O_3 depletion. Triplicate samples (each *c*. 20 mg) of both bryophytes were collected on 3-5 December 1999, when mean O_3 column depth was 205 Dobson units (DU) and 7-9 December 1999, when mean O_3 column depth was 350 DU. Material was immediately frozen at -80 °C and transported back to the UK for analysis. Samples were freeze-dried (Savant Speed Vac Plus, Savant Instruments, Inc., Holbrook, NY, USA), so that pigment concentrations could be calculated relative to sample dry mass, and were added to 5 mL of extraction solvent (80:15:5 mixture of acetone, methanol and water). Samples were ground with a glass rod and incubated overnight at 7 °C in the dark with shaking. The extracts were freeze-dried and 1 mL of injection solvent (70:25:5 mixture of acetone, 0.1 M ammonium acetate and methanol) was immediately added. Samples were shaken for 30 min to dissolve pigments and were passed through 0.45 μm filters. Extracts (60 μL) were diluted 2:1 with ammonium acetate buffer before injection onto a Waters Spherisorb ODS-2 column with a particle size of 5 μm (Alltech Associates, Carnforth, UK). Pigments were separated by reversed phase HPLC at a flow rate of 1 mL min^{-1}. The mobile gradients were solvent A (80:20 mixture of methanol and 0.5 M ammonium acetate), solvent B (90:10 mixture of acetonitrile and water) and solvent C (ethyl acetate) using the protocol described by Wright *et al.* (1991). Methanol, acetonitrile and ethyl acetate HPLC grade reagents were filtered (0.2 μm) and degassed with nitrogen. Water was purified using a Millipore milli-Q system (Millipore, Bedford, MA, USA). Ammonium acetate was AR grade. Pigment detection was at 435, 470 and 665 nm, with spectra from 300-700 nm being collected continuously by a diode array detector (Kontron Instruments Ltd., Watford, UK). U.S. Environmental Protection Agency standards were used to verify the identity of individual pigments using Scientific Committee on Oceanic Research protocols (Jeffrey *et al.* 1997). Pigment derivatives were identified with reference to known HPLC spectra and reference data previously generated using continuous-flow HPLC-MS. To eliminate potential errors owing to differences in extraction efficiencies between samples, concentrations of individual carotenoids were expressed as relative concentrations (percentage of total mass of carotenoids) prior to statistical analyses.

Photochemical yield measurements

A pulse amplitude modulated fluorometer (MINI-PAM photosynthesis yield analyzer, Heinz-Walz GmbH, Effeltrich, Germany) was used to measure minimal chlorophyll *a* fluorescence (F_o) and maximum fluorescence (F_m) induced by a 0.8 s saturating flash on three separate dark-adapted (20 min) colonies of each bryophyte species at solar noon in 1999. F_v/F_m, the maximum quantum yield of photochemistry (in which $F_v = F_m - F_o$), was subsequently calculated (Schreiber *et al.* 1986). A tent was pitched close to the gully from which bryophytes were sampled with an inner enclosure of rubber-backed cloth in which to dark-adapt plants.

O_3 column measurements

Overpass measurements of O_3 column depth (in DU) over Rothera Point were obtained from the Earth Probe Total O_3 Mapping Spectrometer (EP-TOMS), situated aboard the NASA Earth Probe Satellite, through the world wide web (http://jwocky.gsfc.nasa.gov). Data from the spectrometer were not available between 13 and 28 December 1998 and for 17 November in both years.

Irradiance measurements

Global spectral irradiance between 280 and 600 nm (step 0.5 nm) was recorded by a double monochromator grating spectroradiometer (Bentham DM150; Bentham Instruments Ltd., Reading, UK) situated in a laboratory *c.* 500 m from the gully from which bryophytes were sampled. The instrument was calibrated against a 1000 W quartz-halogen tungsten coil filament lamp which had been calibrated relative to National Institute of Standards and Technology standards. The instrument recorded data every 30 min between 08:00 and 19:00 hrs local time in both years and hourly between 20:00 and 07:00 hrs local time in 1999. Data from the spectroradiometer were not available for 25 and 26 November 1998 and for between 19 and 28 December 1998.

Irradiance data were either expressed either as the irradiance (in W m^{-2}) of unweighted UV-B, UV-A or PAR, the irradiance of biologically effective UV-B weighted with the generalized plant action spectrum (UV-B_{BE}; Caldwell 1971) normalized at 300 nm, or the ratio of UV-B to PAR irradiance (hereafter F_{UV-B}/F_{PAR}). Noon values of each parameter were calculated. Integration was also used to calculate daily doses (in kJ m^{-2}) of UV-B_{BE} or of unweighted UV-B, UV-A and PAR from irradiance data. Daily mean F_{UV-B}/F_{PAR} values were also calculated. PAR and F_{UV-B}/F_{PAR} values were under- and overestimated, respectively, relative to most other studies, because irradiances of PAR were recorded up to 600 nm, rather than 700 nm.

Abiotic factor measurements

Ambient temperature (in °C) and relative humidity (%) data were recorded every 5 min in both years by two platinum resistance thermometers (PT100; Labfacility Ltd., Teddington, UK) and a humidity transmitter (Vaisala HMD60UO/YO; Vaisala Inc., Woburn, MA, USA), respectively, under a Stevenson screen situated *c*. 300 m from the gully from which bryophytes were sampled. The temperature of each colony was also recorded during photochemical yield measurements on each day in 1999 with a hand-held digital thermometer with a penetration probe (Checktemp HI98501; Hanna Instruments Inc., Woonsocket, RI, USA).

Data analyses

Irradiance data recorded in 1998 and 1999 were combined and linear regression models were used to determine responses of these data to changes in O_3 column. Pigment data from both years were also combined and stepwise and linear regression models were used to determine the best and other significant predictor variables for pigments from all calculated irradiance and abiotic variables. The best predictor for F_v/F_m was also determined by stepwise linear regression from irradiance parameters and abiotic factors measured in 1999. To determine if there was any lag time in pigment response to O_3 reduction, percentage variance accounted for ($\underline{r}^2$) values were calculated for associations between pigment concentrations and O_3-dependent irradiance parameters measured either at the time of sampling, or at 24 and 48 h prior to sampling of bryophytes. These analyses were made only on individual pigments that apparently responded to O_3 depletion. Daily mean irradiance data were not used in these analyses: noon measurements of those parameters most closely associated with pigment concentrations were used instead. One way ANOVA was used to compare the relative concentrations of individual carotenoids between 3-5 and 7-9 December 1999. Data from the triplicate samples taken each day for HPLC analyses were pooled prior to analysis. Differences in O_3 column depth, irradiance parameters and abiotic factors between 3-5 and 7-9 December 1999 were also determined by one way ANOVA.

Multiple regression models were used to determine the effects on F_v/F_m of colony temperature, UV-B screening pigments, carotenoids and the irradiance parameter associated with O_3 depletion which was most consistently associated with pigment concentrations. Three- and four-term models were used into which predictor variables were fitted sequentially. This process enabled the construction of an analysis of variance table in which the effects of each variable, and of different sequences of variables, on F_v/F_m were determined through examination of F-values (Draper & Smith 1998). Temperature was fitted first in all models to remove the effect of this variable on F_v/F_m; subsequent terms (themselves inter-correlated) were fitted in different orders to examine the effects that these had on the relative significance of other predictors. Statistical analyses were conducted in the MINITAB 13.1 and GENSTAT 5.4.1 packages.

Results

Variation in O_3 column depth, temperature and radiative environment

Data from the EP-TOMS indicated there was wide variation in O_3 column depth over Rothera Point during the period of study (Fig. 1). Maximum and minimum O_3 column depths were 370 and 192 DU, recorded on 19 November 1998 and 4 December 1999. Air temperatures recorded in 1999 were between -1.5 and 1.9 °C: colony temperatures were up to 18.5 °C higher than these (Fig. 1, inset). Linear regression, using O_3 column depth as a predictor variable, and the calculated irradiance parameters as responses, indicated that there were no significant ($P<0.05$) associations between O_3 column depth and the irradiances or daily doses of UV-A or PAR received at ground level. However, a strong inverse association between O_3 column depth and daily mean $F_{UV\text{-}B}/F_{PAR}$ was recorded (Fig. 2a). Owing to the influence of clouds on the penetration of UV-B radiation to the Earth's surface, a weaker, but significant, inverse association between O_3 column depth and the daily dose of unweighted UV-B radiation was also found (Fig. 2b). There were also inverse associations between O_3 column depth and noon $F_{UV\text{-}B}/F_{PAR}$, the daily dose and noon irradiance of UV-B_{BE}, and the noon irradiance of unweighted UV-B (Table 1). Minimum, maximum and mean daily doses of UV-B_{BE} were 0.9, 10.3 and 4.1 kJ m^{-2}, respectively.

Variation in pigmentation

Regression analyses indicated that none of the irradiance parameters associated with O_3 depletion were significant predictors for concentrations of chlorophylls *a* and *b* or total chlorophyll extracted from tissues of either *Cephaloziella* or *Sanionia* (regression summary; $\underline{r}^2$ = 2 - 6%, all $P>0.05$). However, stepwise linear regression indicated that two irradiance parameters which were directly associated with O_3 depletion were best predictors for concentrations of UV-B screening pigments and total carotenoids extracted from bryophyte tissues. The best predictor for concentrations of UV-B screening pigments in *Cephaloziella* was daily UV-B dose (Fig. 3a). Significant associations were also recorded between concentrations of UV-B screening pigments in *Cephaloziella* tissues and each of the other five irradiance parameters associated with O_3 depletion (Table 2). The best predictor for UV-B screening pigments in *Sanionia* was daily mean $F_{UV\text{-}B}/F_{PAR}$ (Fig. 3b). Noon $F_{UV\text{-}B}/F_{PAR}$ and daily dose and noon irradiance of UV-B_{BE} were also associated with concentrations of UV-B screening pigments in this species (Table 2). The best predictor for concentrations of carotenoids in both bryophyte species was daily mean $F_{UV\text{-}B}/F_{PAR}$ (Fig. 4a, b). Noon $F_{UV\text{-}B}/F_{PAR}$ was also significantly associated with carotenoid concentrations in *Cephaloziella* tissues. The ratio of UV-B_{BE} to PAR irradiance was not a better predictor for pigment concentrations than daily UV-B dose and F_{UV-B}/F_{PAR} (data not shown).

The lag time in the accumulation of pigments in response to O_3 depletion appeared to be < 24 h: the highest r^2 values for associations between pigment concentrations and O_3-dependent irradiance parameters were recorded when pigment concentrations were compared with irradiance data measured at the time of sampling. Relative to associations between pigment concentrations and irradiance parameters recorded at the time of sampling, there were 46% and 70% reductions in r^2 values as concentrations of UV-B screening pigments in *Cephaloziella* were compared with noon irradiance of UV-B measured 24 and 48 h prior to sampling (Fig. 5a). For carotenoids in *Cephaloziella*, 50% and 67% reductions in r^2 values occurred as pigment concentrations were compared with F_{UV-B}/F_{PAR} measured 24 and 48 h before sampling, respectively (Fig. 5b), and in *Sanionia*, there were 19% and 31% reductions in r^2 values as UV-B screening pigment concentrations were compared with F_{UV-B}/F_{PAR} recorded 24 and 48 h prior to sampling (Fig. 5c). It was not possible to determine the lag time in the response of carotenoids in *Sanionia* because concentrations of these pigments in this species were only significantly associated with daily mean F_{UV-B}/F_{PAR} (Table 2).

One way ANOVA indicated a significant 41% decrease in daily mean F_{UV-B}/F_{PAR} between 3-5 and 7-9 December 1999 (0.0070 *v*. 0.0041, respectively, $F_{1,5} = 103.0$, $P<0.001$). Noon F_{UV-B}/F_{PAR} also showed a similar 40% decrease between these two time periods ($F_{1,5} = 86.4$, $P<0.001$). No other significant differences in any other irradiance parameters or abiotic factors were recorded between 3-5 and 7-9 December 1999. HPLC analyses of individual carotenoids indicated that there were significant differences in the relative concentrations of individual carotenoids in *Cephaloziella* and *Sanionia* tissues between 3-5 and 7-9 December. The relative concentration of neoxanthin increased in tissues of both species in apparent response to O_3 depletion, whilst in *Cephaloziella*, violaxanthin concentration increased and both lutein and zeaxanthin concentrations decreased (Table 3). In *Sanionia*, the concentration of β,β-carotene increased substantially under depleted O_3 column (Table 3). Relative concentrations of antheraxanthin, and carotenoids not extracted from tissues of both *Cephaloziella* or *Sanionia* (α- and β,ε-carotene and their derivatives, canthaxanthin and one unidentified carotenoid) did not apparently respond to O_3 depletion.

Variation in photochemical yield

Minimum F_v/F_m values (± SD) recorded were 0.105 (± 0.020) and 0.210 (± 0.015) for *Cephaloziella* and *Sanionia*, respectively, both of which were measured on 11 November 1999. Maximum F_v/F_m values for

Cephaloziella and *Sanionia* were 0.673 (± 0.026) and 0.674 (± 0.013), recorded on 6 and 9 December 1999, respectively. Univariate linear regression models indicated that there were no direct associations between F_v/F_m and O_3-dependent irradiance parameters in either *Cephaloziella* or *Sanionia* (regression summary; $\underline{r}^2$ = 0.4 - 12%, all P>0.05). Stepwise linear regression analyses indicated that colony temperature was the best predictor for F_v/F_m in both species and that it accounted for 62% and 59% of the variance in F_v/F_m of *Cephaloziella* and *Sanionia*, respectively (both P<0.001).

Multiple regression models similarly indicated a strong effect of colony temperature on F_v/F_m (Table 4). When $F_{UV\text{-}B}/F_{PAR}$ was entered into three-term multiple regression models along with temperature and concentrations of UV-B screening pigments, 76.5% and 65.8% of the variances in F_v/F_m of *Cephaloziella* and *Sanionia* were accounted for, respectively. Although univariate analyses showed no effect of $F_{UV\text{-}B}/F_{PAR}$ on F_v/F_m, in both species, $F_{UV\text{-}B}/F_{PAR}$ was inversely correlated with F_v/F_m in multiple regression models. When $F_{UV\text{-}B}/F_{PAR}$ was fitted before UV-B screening pigments in three-term models, we found significant (P<0.01) effects of both variables on F_v/F_m. However, when UV-B screening pigments were fitted before $F_{UV\text{-}B}/F_{PAR}$, there were highly significant positive effects of the pigments on F_v/F_m, which removed the significant influence of $F_{UV\text{-}B}/F_{PAR}$ on F_v/F_m in both species (Table 4).

When carotenoid concentration (estimated by spectrophotometric analyses) was entered into four-term models along with colony temperature, UV-B screening pigments and $F_{UV\text{-}B}/F_{PAR}$, 76.6% and 67.1% of the variances in F_v/F_m of *Cephaloziella* and *Sanionia* were accounted for, respectively. Carotenoids did not explain a significant amount of variation in F_v/F_m of *Cephaloziella* when fitted before or after either $F_{UV\text{-}B}/F_{PAR}$ or UV-B screening pigments (Table 4). However, in *Sanionia*, carotenoids explained a significant amount of variation in F_v/F_m only when fitted before UV-B screening pigments (Table 4). In common with the results from the three-term models, when $F_{UV\text{-}B}/F_{PAR}$ was entered before UV-B screening pigments, significant effects of both variables on F_v/F_m were found, but when the order in which these two variables were fitted was reversed, significant effects of UV-B screening pigments remained, which removed the significant effect of $F_{UV\text{-}B}/F_{PAR}$ on F_v/F_m in both species. Moreover, for *Sanionia*, when carotenoids were fitted before UV-B screening pigments and $F_{UV\text{-}B}/F_{PAR}$, there were highly significant positive effects of both pigments on F_v/F_m, which removed the significant influence of $F_{UV\text{-}B}/F_{PAR}$ on F_v/F_m (Table 4).

Discussion

This study took place in an environment with no local pollution sources and hence no tropospheric O_3 pollution. All variation in O_3 column depth over the study site can hence be assumed to have taken place in the stratosphere as a result of photochemical catalysis of the gas by chlorine and bromine species. Our study therefore suggests that *Cephaloziella* and *Sanionia* responded rapidly to changes in the spectral environment arising from stratospheric O_3 depletion by increasing the synthesis of UV-B screening pigments and carotenoids in foliage. We are unaware of any other studies in the literature that have demonstrated significant associations between changes in the spectral environment consequent on stratospheric O_3 depletion and the pigmentation of terrestrial plants.

Our analyses suggested that the time taken for pigments to respond to O_3 depletion was < 24 h. That Antarctic bryophytes apparently respond rapidly to O_3 depletion is perhaps surprising, given the temperature-dependence of reactions in metabolic pathways, but the temperatures of bryophyte colonies in our study reached 20 °C on cloudless days, which would have enabled rapid response to O_3 depletion. At present it is unclear at what rate UV-B screening pigments are synthesized and decomposed in plant tissues in the natural environment: laboratory studies on temperate plant species under low PAR irradiances indicate that the synthesis of UV-B screening pigments is induced within several hours of exposure to UV-B radiation (Jordan *et al.* 1994), owing to the induction of genes encoding chalcone synthase, a key enzyme in the flavonoid biosynthesis pathway (Hahlbrock & Scheel 1989). Similarly, a field study on the alpine fern *Cryptogramma crispa* indicated that UV-B screening pigments are synthesized within 12 h of exposure to full sunlight, relative to plants under UV-B absorbing screens (Veit *et al.* 1996). However, laboratory studies on excised hypocotyls of crop plants indicate that flavonoids do not undergo rapid decomposition (Margna & Vainjärv 1981) and glasshouse experiments indicate that concentrations of flavonoid glycosides and aglycones in *Betula pendula* tissues may also take several days to respond to UV-B radiation, although those of condensed tannins may respond within 24 h (Lavola *et al*. 2000).

In a comprehensive review of plant responses to elevated UV-B radiation, Searles *et al.* (2001), using a meta-analysis of data from 62 outdoor irradiation experiments, concluded that the accumulation of UV-B screening pigments in foliage was the most consistent response of plants to elevated UV-B. Our data confirm that this response can be demonstrated *in situ*. Field-based experiments in Antarctica have similarly shown changes in UV-B screening pigments in response to altered UV-B radiation: Ruhland & Day (2000) found that exposure of the pearlwort *Colobanthus quitensis* to near-ambient solar UV-B radiation for four months at Stepping Stones Island (64° S) on the western Antarctic Peninsula led to increased concentrations of UV-B screening pigments in foliage, compared with plants exposed to reduced UV-B radiation. Foliage of *C. quitensis* and the grass *Deschampsia antarctica* also accumulated higher concentrations of UV-B screening pigments after two months of exposure to near-ambient UV-B radiation than plants exposed to reduced UV-B at a location close to Stepping Stones Island (Xiong & Day 2001). In addition, increased synthesis of UV-B screening pigments in response to O_3 depletion in tissues of the moss *Andreaea regularis* have also been recorded at Rothera Point during late austral spring and early summer 1998 (K. Newsham, unpubl.).

Although Searles *et al.* (2001) found no effect of elevated UV-B radiation on concentrations of carotenoids, in our study, we found loose, but significant, associations between $F_{UV\text{-}B}/F_{PAR}$ and carotenoid concentrations in both bryophytes. Increased concentrations of carotenoids have also been shown to be present in the foliage of *C. quitensis* and *D. antarctica* exposed to near-ambient UV-B radiation compared to foliage of plants exposed to reduced UV-B by Xiong & Day (2001). Increased synthesis of carotenoids in *A. regularis* tissues has also been recorded in response to O_3 depletion over Rothera Point (K. Newsham, unpubl.). Similarly, carotenoid concentrations in Southern Ocean phytoplankton increase during periods of O_3 depletion (Smith *et al.* 1992), and empirical studies in artificial systems have also shown that exposure of plants to elevated UV-B radiation increases carotenoid concentrations in foliage (e.g. Middleton & Teramura 1993). In contrast, Gehrke (1998; 1999), using fluorescent UV lamps in sub-Arctic heaths and bogs, showed that exposure to UV-B radiation simulating a 15% depletion of ozone decreased concentrations of carotenoids in tissues of *Sphagnum fuscum* and *Polytrichum commune*.

In addition to recording increased total carotenoid concentrations in bryophyte tissues in response to O_3 depletion, our study suggested that changes in $F_{UV\text{-}B}/F_{PAR}$ associated with O_3 column depth also influenced the relative concentrations of specific carotenoids. An increase in neoxanthin concentration was the only response observed in both bryophyte species to O_3 depletion. Other responses differed between the two bryophyte species: two xanthophyll cycle pigments, violaxanthin and zeaxanthin, respectively increased and decreased in *Cephaloziella* tissues during a period of O_3 depletion. Although the diurnal synthesis of xanthophyll cycle pigments is well known to be associated with PAR levels (Demmig-Adams & Adams 1996), the influence of UV-B radiation on these carotenoids is less well documented. However, reduced zeaxanthin concentration in response to elevated UV-B has been demonstrated in the Antarctic alga *Leptosomia simplex* under controlled conditions (Dohler 1998). We also recorded decreased lutein concentrations in *Cephaloziella* under depleted O_3 column, which is also corroborated by Dohler (1998), who found reduced concentrations of this carotenoid in *L. simplex* tissues exposed to elevated UV-B. The substantial increase in the concentration of β,β-carotene in *Sanionia* tissues observed in our study is also corroborated by the observations of Underwood *et al.* (1999), who found increased concentrations of this carotenoid in a diatom mat exposed to elevated UV-B radiation.

Although we recorded an association between concentrations of UV-B screening pigments in tissues of *Cephaloziella* and daily unweighted UV-B dose, consistent associations were found between pigment concentrations and $F_{UV\text{-}B}/F_{PAR}$. These findings confirm the view expressed by other authors that the relative irradiance of UV-B to PAR is an important determinant of plant response to simulated O_3 depletion in artificial irradiation systems (Middleton & Teramura 1993; Caldwell & Flint 1994; Allen *et al.* 1998). However, with the exception of the study by Caldwell *et al.* (1994), there are few data to support the argument that relative irradiance of UV-B to PAR directly influences plant response in the field. Caldwell *et al.* (1994) found that the growth of *Glycine max* responded most severely to elevated UV-B simulating a 36% reduction in O_3 column only when UV-A and PAR were reduced to 50% of their fluxes in solar radiation. Our data, which show increased synthesis of UV-B screening pigments in *Sanionia* as $F_{UV\text{-}B}/F_{PAR}$ increased, corroborate the data of Caldwell *et al.* (1994), who found the highest concentrations of UV-B screening pigments extracted per unit of *G. max* leaf mass in plants exposed to elevated UV-B radiation and reduced UV-A and PAR.

No direct associations between any of the irradiance parameters associated with O_3 depletion and F_v/F_m of either bryophyte species were recorded in our study. This corroborates data from Searles *et al.* (2001), and other studies in artificial irradiation systems (Caldwell & Flint 1994), that have shown elevated UV-B to have no influence on this parameter. These data suggest that the primary production of these two bryophyte species is unaffected by O_3 depletion, and corroborate the view expressed by other authors that O_3 reduction does not influence the photosynthetic competence of plants (Fiscus & Booker 1995; Allen *et al.* 1998). The lack of association between O_3-dependent irradiance parameters and chlorophyll concentrations in both bryophytes corroborates data from many other plant species in outdoor irradiation systems (Searles *et al.* 2001) and Antarctic field studies (Day *et al.* 1999; Xiong & Day 2001), and further supports the argument that the primary production of *Cephaloziella* and *Sanionia* is unaffected by stratospheric O_3 depletion.

Although we could find no direct associations between O_3-dependent irradiance parameters and F_v/F_m, we found a strong positive effect of colony temperature on F_v/F_m, confirming previous observations that this parameter is sensitive to low temperatures (Bolhàr-Nordenkampf & Öquist 1993). Once the influence of temperature on F_v/F_m was removed, we found that fitting UV-B screening pigments before $F_{UV\text{-}B}/F_{PAR}$ removed the significant negative influence of the latter variable on F_v/F_m. This analysis implied that UV-B screening pigments had a protective effect on F_v/F_m and that the paucity of effect of $F_{UV\text{-}B}/F_{PAR}$ on the photochemical yield of *Cephaloziella* and *Sanionia* was owing to increased synthesis of UV-B screening pigments. Furthermore, fitting carotenoids before UV-B screening pigments and $F_{UV\text{-}B}/F_{PAR}$ in *Sanionia*, the species with the lowest concentrations of UV-B screening pigments, again removed the negative influence of $F_{UV\text{-}B}/F_{PAR}$ on F_v/F_m. These analyses suggested a weak, but significant, protective influence of carotenoids on photosynthesis during periods of O_3 depletion in this species and corroborate the view that carotenoids have a subsidiary role to UV-B screening pigments in protecting plants from UV-B radiation (Cockell & Knowland 1999). Previous studies under controlled conditions have similarly concluded that UV-B screening pigments and carotenoids may protect the photosynthesis and growth of plants from elevated UV-B. For example, Li *et al.* (1993), using a mutant of *Arabidopsis thaliana* with an impaired ability to synthesize flavonoids, showed significant reductions in the dry mass accumulation of mutant plants exposed to elevated UV-B radiation, compared with wild type plants. Similarly, the growth of carotenoid deficient mutants of *Glycine max* has been shown to be reduced by elevated UV-B radiation, relative to wild type plants (Middleton & Teramura 1993). Flavonoid deficient mutants of *Hordeum vulgare*, containing only 7% of total extractable flavonoids in primary leaves compared with the mother variety, also showed significant reductions in F_v/F_m when exposed to elevated UV-B in growth chambers (Reuber *et al.* 1996). A different approach was used by Reuber *et al.* (1993), who treated *Secale cereale* with an inhibitor of phenylalanine ammonium lyase to reduce flavonoid production. Plants treated with the inhibitor exhibited reduced F_v/F_m when compared with control plants with normal levels of flavonoids after exposure to elevated UV-B radiation under controlled conditions.

In conclusion, the data presented here indicate that changes to the radiative environment associated with stratospheric O_3 depletion elicit responses in the pigmentation of terrestrial plants. They suggest that two Antarctic bryophytes responded rapidly to O_3-dependent increases in solar UV-B by synthesizing UV-B screening pigments and carotenoids in tissues, and that pigmentation may have conferred protection from potentially deleterious effects of UV-B radiation on photochemical yield, even during periods of intense O_3 depletion. Future research is required to determine whether or not similar responses occur in other plant species, and to address the potential physiological costs to plants of synthesizing and maintaining photoprotective compounds in foliage.

Acknowledgments

This work was funded by the United Kingdom Natural Environment Research Council. O_3 data were supplied *gratis* by the NASA/GSFC TOMS O_3 Processing Team. Staff of the Netherlands Institute of Ecology loaned the PAM fluorometer for use in 1999. Paul Geissler and Andrew Rossaak maintained the Bentham spectroradiometer at Rothera research station and other staff at the station provided logistic support. Andy Clarke and two anonymous referees provided comments on the manuscript and Martyn Caldwell and colleagues provided preprints of papers. All are gratefully acknowledged.

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Table 1 Data from linear regression analyses showing associations between O_3 column depth (measured by EP-TOMS) and irradiance parameters recorded at ground level at Rothera Point

Predictor variable	Response variable	$\bar{r}^2$	Coefficient	$F_{1,51}$	P-value
O_3 column depth	Noon $F_{UV\text{-}B}/F_{PAR}$	77.0	-2.1×10^{-5}	170.95	< 0.001
	Daily dose of UV-B_{BE}	45.4	-2.4×10^{-2}	42.39	< 0.001
	Noon irradiance of UV-B_{BE}	33.3	-8.0×10^{-4}	25.41	< 0.001
	Noon irradiance of UV-B	15.5	-2.4×10^{-3}	9.34	0.004

Note: See Materials and methods for details of how data were expressed in analyses.

Table 2 Data from linear regression analyses showing associations between irradiance parameters associated with O_3 depletion and concentrations of UV-B screening pigments and carotenoids in *Cephaloziella* and *Sanionia* tissues

Response variable	Predictor variable	$\bar{r}^2$	Coefficient	F-value	P-value
Cephaloziella					
UV-B s.p.	Daily dose of UV-B_{BE}	25.5	1.5×10^2	19.48	< 0.001
	Noon irradiance of UV-B_{BE}	21.3	3.5×10^3	15.46	< 0.001
	Noon irradiance of UV-B	19.4	7.5×10^2	13.75	< 0.001
	Daily mean $F_{UV\text{-}B}/F_{PAR}$	14.8	1.9×10^5	9.90	0.003
	Noon $F_{UV\text{-}B}/F_{PAR}$	8.6	1.3×10^5	5.36	0.024
Carotenoids	Noon $F_{UV\text{-}B}/F_{PAR}$	15.0	2.1×10^1	9.90	0.003
Sanionia					
UV-B s.p.	Noon $F_{UV\text{-}B}/F_{PAR}$	17.1	8.2×10^4	11.95	0.001
	Daily dose of UV-B_{BE}	7.5	3.5×10^1	4.69	0.035
	Noon irradiance of UV-B_{BE}	6.7	8.8×10^2	4.16	0.046

Note: UV-B s.p. = UV-B screening pigments. Total d.f. in analyses on *Cephaloziella* and *Sanionia* were 57 and 58, respectively. See Materials and methods for details of how data were expressed in analyses.

Table 3 Relative concentrations of individual carotenoids in *Cephaloziella* and *Sanionia* tissues under normal and depleted O_3 columns (350 and 205 DU, respectively). Data are mean (± SD) relative concentrations (percentage of total mass of carotenoids) of each pigment

	Cephaloziella				*Sanionia*			
	Concentration (%)				Concentration (%)			
	Normal O_3	Depleted O_3	$F_{1,5}$	*P*-value	Normal O_3	Depleted O_3	$F_{1,5}$	*P*-value
Neoxanthin	10.3 (± 2.31)	14.4 (± 0.91)	8.1	0.047	9.63 (± 0.40)	13.45 (± 0.36)	99.1	< 0.001
Violaxanthin	0.46 (± 0.79)	6.15 (± 0.94)	63.6	0.001	3.07 (± 0.32)	3.88 (± 0.75)	2.9	0.162
Lutein	76.07 (± 1.46)	68.80 (± 1.99)	26.0	0.007	60.16 (± 6.04)	57.79 (± 7.68)	0.2	0.696
Zeaxanthin	8.03 (± 1.26)	5.79 (± 0.44)	8.5	0.043	14.22 (± 3.02)	12.46 (± 2.93)	0.5	0.508
β,β-carotene	0.59 (± 1.01)	0.26 (± 0.45)	0.3	0.639	0.01 (± 0.01)	6.09 (± 3.75)	7.9	0.048

Note: Data for antheraxanthin, and carotenoids not extracted from both species, are not shown in Table.

Table 4 Data from three- and four-term stepwise multiple regression models using F_v/F_m of *Cephaloziella* and *Sanionia*, measured in 1999, as response variables. Note that, for clarity, the effects of colony temperature on F_v/F_m are shown only once for three- and four-term models

			Cephaloziella F_v/F_m		*Sanionia* F_v/F_m	
No. terms in model	Model run	Predictor variable	$F_{1,27}$	P-value	$F_{1,27}$	P-value
Three	1	Colony temperature	71.56	< 0.001	27.05	< 0.001
		F_{UV-B}/F_{PAR}	8.75	0.007	16.69	< 0.001
		UV-B s.p.	9.64	0.005	11.45	0.002
	2	UV-B s.p.	18.34	< 0.001	28.14	< 0.001
		F_{UV-B}/F_{PAR}	0.05	0.823	0.00	0.969
Four	1	Colony temperature	72.01	< 0.001	27.99	< 0.001
		F_{UV-B}/F_{PAR}	8.81	0.007	17.27	< 0.001
		UV-B s.p.	9.70	0.005	11.85	0.002
		Carotenoids	1.15	0.294	1.87	0.185
	2	F_{UV-B}/F_{PAR}	8.81	0.007	17.27	< 0.001
		Carotenoids	0.00	0.948	7.33	0.012
		UV-B s.p.	10.84	0.003	6.39	0.018
	3	UV-B s.p.	18.45	< 0.001	29.12	< 0.001
		Carotenoids	1.20	0.285	1.86	0.185
		F_{UV-B}/F_{PAR}	0.00	0.948	0.01	0.942
	4	Carotenoids	1.78	0.196	20.24	< 0.001
		UV-B s.p.	17.87	< 0.001	10.73	0.003
		F_{UV-B}/F_{PAR}	0.00	0.948	0.01	0.942

Note: UV-B s.p. = UV-B screening pigments.

Figure legends for Newsham *et al.*

Fig. 1 Variation in O_3 column depth over Rothera Point during the period of study. *Inset*: air (❍) and colony (•) temperatures in 1999. Colony temperatures are means of measurements on both bryophyte species.

Fig. 2 Daily mean (a) ratio of UV-B irradiance to PAR irradiance ($F_{UV\text{-}B}/F_{PAR}$) and (b) daily unweighted UV-B dose received at ground level as a function of O_3 column depth over Rothera Point. Regression details: (a) r^2 = 87.3%, coefficient = -2.0×10^{-5}, $F_{1,23}$ = 350.40, P<0.001; (b) r^2 = 24.2%, coefficient = -8.2×10^{-2}, $F_{1,23}$ = 16.28, P<0.001. Note that axes do not extend to zero.

Fig. 3 Mean concentrations of UV-B screening pigments in acidified methanol extracts, expressed as the area under the absorbance curve in the range 280-315 nm, ($AUC_{280\text{-}315}$) g^{-1} dry mass of (a) *Cephaloziella* tissue as a function of unweighted daily UV-B dose and (b) *Sanionia* tissue as a function of daily mean $F_{UV\text{-}B}/F_{PAR}$. Values are means of three replicates ± SD. Regression details: (a) r^2 = 25.8%, coefficient = 3.0×10^1, $F_{1,57}$ = 19.81, P<0.001; (b) r^2 = 19.1%, coefficient = 9.9×10^4, $F_{1,58}$ = 13.69, P<0.001. Note that axes do not extend to zero.

Fig. 4 Mean mass of total carotenoids g^{-1} dry mass of (a) *Cephaloziella* and (b) *Sanionia* tissue as a function of daily mean $F_{UV\text{-}B}/F_{PAR}$. Values are means of three replicates ± SD. Regression details: (a) r^2 = 18.6%, coefficient = 2.8×10^1, $F_{1,57}$ = 12.82, P<0.001; (b) r^2 = 7.7%, coefficient = 2.9×10^1, $F_{1,58}$ = 4.86, P = 0.031. Note that axes do not extend to zero.

Fig. 5 Percentage variance accounted for (r^2) values for associations between O_3-dependent irradiance parameters measured 0, 24 and 48 h before sampling and (a) *Cephaloziella* UV-B screening pigments, (b) *Cephaloziella* total carotenoids and (c) *Sanionia* UV-B screening pigments. Irradiance parameters were noon irradiance of unweighted UV-B in (a) and noon $F_{UV\text{-}B}/F_{PAR}$ in (b) and (c). Asterisks denote significance of associations (***; P<0.001, **; P<0.01, *; P<0.05 and n.s.; P>0.05).

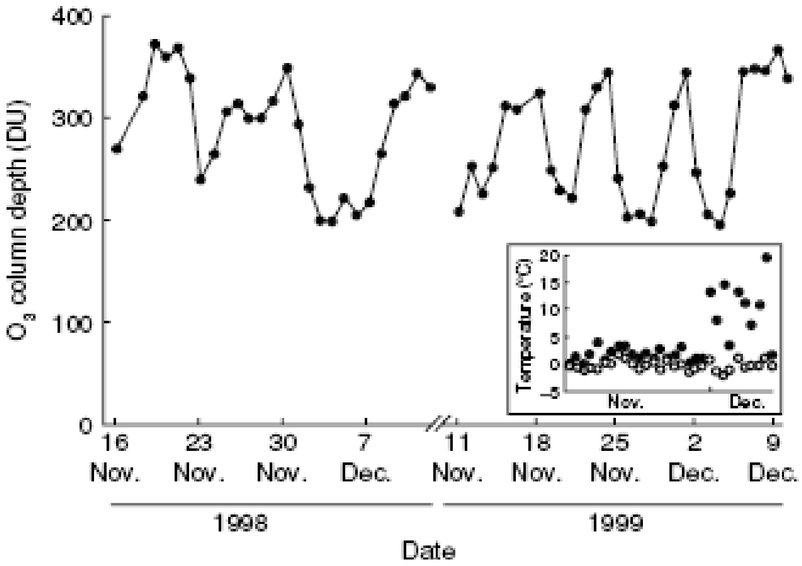
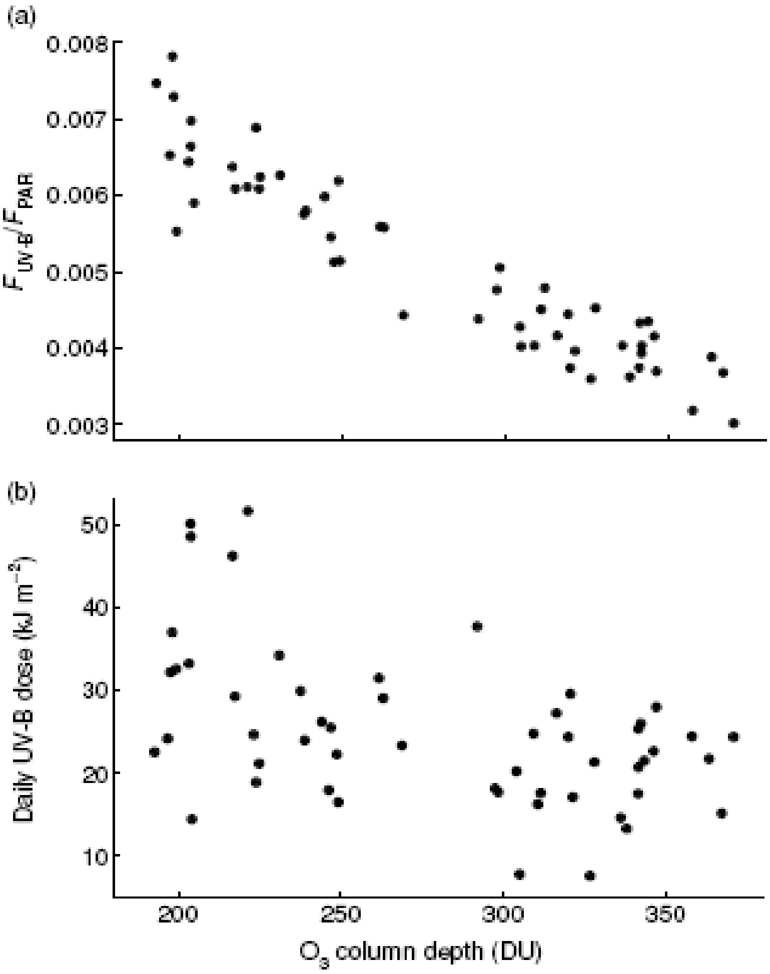

400
300
200
100
0
O_3 column depth (DU)
16 Nov.
23 Nov.
30 Nov.
7 Dec.
11 Nov.
18 Nov.
25 Nov.
2 Dec.
9 Dec.
1998
1999
Date
Temperature (°C)
20
15
10
5
0
−5
Nov.
Dec.

Fig. 1



(a)
0.008
0.007
0.006
0.005
0.004
0.003
F_{UV-B}/F_{PAR}
(b)
50
40
30
20
10
Daily UV-B dose (kJ m^{-2})
200
250
300
350
O_3 column depth (DU)

Fig. 2

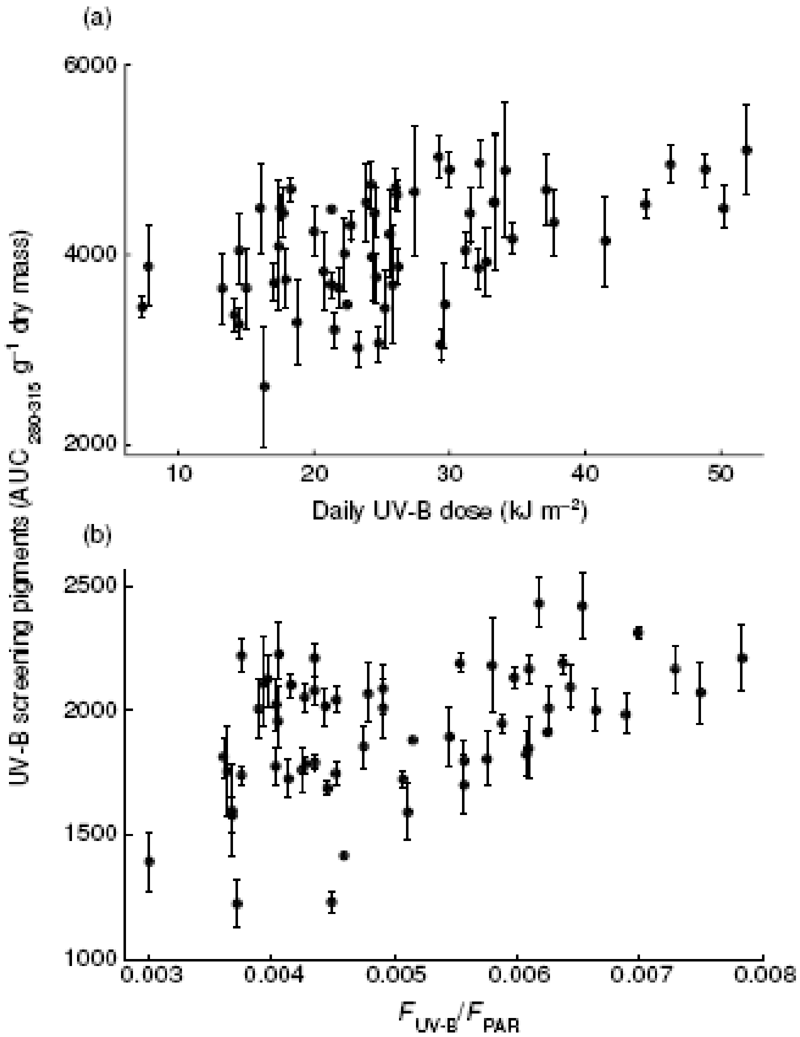

(a)
6000
4000
2000
10
20
30
40
50
Daily UV-B dose (kJ m⁻²)
UV-B screening pigments (AUC280-315 g⁻¹ dry mass)
(b)
2500
2000
1500
1000
0.003
0.004
0.005
0.006
0.007
0.008
FUV-B/FPAR

Fig. 3

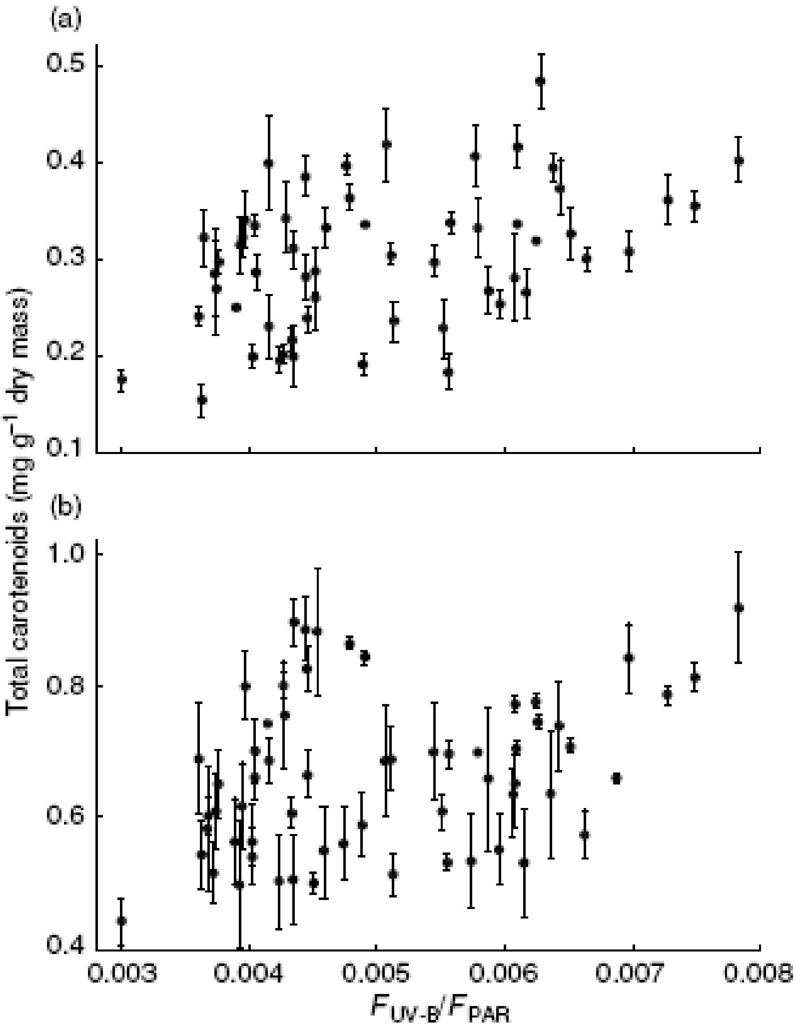

(a)
(b)
Total carotenoids (mg g^{-1} dry mass)
$F_{UV\text{-}B}/F_{PAR}$
0.1
0.2
0.3
0.4
0.5
0.4
0.6
0.8
1.0
0.003
0.004
0.005
0.006
0.007
0.008

Fig. 4

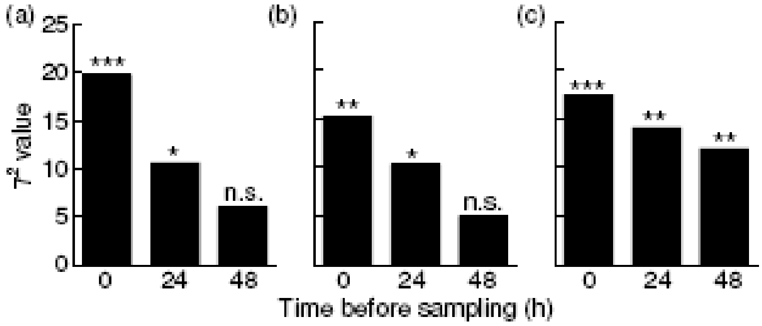

(a)
(b)
(c)
25
20
15
10
5
0
T^2 value
***
*
n.s.
**
*
n.s.
***
**
**
0
24
48
0
24
48
0
24
48
Time before sampling (h)

Fig. 5